Essential roles of 11 β -HSD1 in regulating brown adipocyte function

Juan Liu^{1,*}, Xiaocen Kong^{1,*}, Long Wang^{1,*}, Hanmei Qi¹, Wenjuan Di¹, Xiao Zhang³, Lin Wu¹, Xia Chen¹, Jing Yu¹, Juanmin Zha¹, Shan Lv¹, Aisen Zhang¹, Peng Cheng¹, Miao Hu¹, Yujie Li¹, Jianhua Bi¹, Yan Li⁵, Fang Hu⁵, Yi Zhong⁴, Yong Xu² and Guoxian Ding¹

¹Department of Gerontology, First Hospital Affiliated to Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, People's Republic of China

²Departments of Pathophysiology, Nanjing Medical University, Nanjing, People's Republic of China

³Preventive Medicine, Feinberg School of Medicine Northwestern University, Chicago, Illinois, USA

⁴Department of Pharmaceutical Chemistry, China Pharmaceutical University, Nanjing, People's Republic of China

⁵Metabolic Syndrome Research Center of Central South University, Institute of Metabolism and Endocrinology, the Second Xiangya Hospital of Central South University, Changsha, People's Republic of China

*(J Liu, X Kong and L Wang contributed equally to this work.)

Correspondence should be addressed to G Ding
Email: dinggx@njmu.edu.cn

Abstract

Brown adipose tissue (BAT) increases energy expenditure and is an attractive therapeutic target for obesity. 11 β -Hydroxysteroid dehydrogenase type 1 (11 β -HSD1), an amplifier of local glucocorticoid activity, has been shown to modulate white adipose tissue (WAT) metabolism and function. In this study, we investigated the roles of 11 β -HSD1 in regulating BAT function. We observed a significant increase in the expression of BAT-specific genes, including *UCP1*, *Cidea*, *Cox7a1*, and *Cox8b*, in BVT.2733 (a selective inhibitor of 11 β -HSD1)-treated and 11 β -HSD1-deficient primary brown adipocytes of mice. By contrast, a remarkable decrease in BAT-specific gene expression was detected in brown adipocytes when 11 β -HSD1 was overexpressed, which effect was reversed by BVT.2733 treatment. Consistent with the *in vitro* results, expression of a range of genes related to brown fat function in high-fat diet-fed mice treated with BVT.2733. Our results indicate that 11 β -HSD1 acts as a vital regulator that controls the expression of genes related to brown fat function and as such may become a potential target in preventing obesity.

Key Words

- 11 β -hydroxysteroid dehydrogenase type 1
- BVT.2733
- brown adipose tissue
- fatty β -oxidation
- obesity

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Introduction

Obesity has become a global epidemic with complications substantially contributing to health care costs and mortality (Stryjecki & Mutch 2011). Obesity is the condition characterized by excessive accumulation of white fat cells, which are specialized in energy preservation in the form of triglycerides. However, adipose tissue also contains another type of fat cells called 'brown adipocyte'. By contrast to white adipose tissue (WAT), brown adipose tissue (BAT) plays an active role in energy expenditure and fatty acid (FA) oxidization for

heat dissipation (Cannon & Nedergaard 2008). Recent evidence has demonstrated that BAT is critically important for energy equilibrium (Cypess & Kahn 2010). For example, increase in BAT in mice could promote energy expenditure, reduce adiposity, and protect mice from diet-induced obesity (Almind *et al.* 2007). Furthermore, BAT ablation in mice increased obesity in response to high-fat diets (HFD; Tateishi *et al.* 2009). Therefore, BAT represents an ideal target for the modulation of energy expenditure.

BAT has previously been considered to be important only in small mammals and human infants for body temperature maintenance with minimal physiological relevance in adults. However, recent studies using PET/CT scan techniques have revealed the presence of metabolically active BAT in human adults (Cypess *et al.* 2009, van Marken Lichtenbelt *et al.* 2009, Virtanen *et al.* 2009). BAT may thus play a much more important role in human metabolism than previously appreciated. Promotion of BAT function in human would offer the possibility of increasing energy expenditure and protection against obesity (Seale & Lazar 2009, Orava *et al.* 2011).

11 β -Hydroxysteroid dehydrogenase type 1 (11 β -HSD1), a key enzyme converting inactive glucocorticoid into bioactive forms, is considered as a crucial amplifier of glucocorticoid activity in peripheral tissues (Seckl 2004). Studies have shown that 11 β -HSD1 was a powerful regulator in modulating WAT metabolism and function (Bujalska *et al.* 1999, Stimson & Walker 2007). Inhibition of 11 β -HSD1 could prevent the differentiation of white adipocyte, reduce white adipose accumulation, and improve the function of WAT (London & Castonguay 2009, Staab & Maser 2010). Besides, we recently found that BVT.2733, a selective 11 β -HSD1 inhibitor, could even attenuate the inflammation of WAT in diet-induced obese mice (Wang *et al.* 2012). However, the role of 11 β -HSD1 in BAT remains largely unknown.

In this study, we investigated the role of 11 β -HSD1 in brown fat function using murine primary brown adipocyte *in vitro* and in obese mice *in vivo*. Our data demonstrated that 11 β -HSD1 loss-of-function in brown adipocyte resulted in increased, whereas 11 β -HSD1 gain-of-function led to diminished, expression of brown fat functional genes. Our data suggest that 11 β -HSD1 may exert a negative effect on brown fat function. Therefore, targeting 11 β -HSD1 may promote energy expenditure mediated by brown adipocyte and potentially reduce health risks imposed by obesity.

Materials and methods

Plasmids and lentiviral vectors

Full-length mouse cDNA sequence for 11 β -HSD1 was cloned into pLL3.7-bsd lentiviral vector. The RNAi construct for 11 β -HSD1 was generated using one sequence in the coding region of 11 β -HSD1: 5'-CCGGGCACTATGGAAGACATGACATCTCGAGATGTCATGTCTTCCATAGTGCTTTTGTG-3' (Sigma-Aldrich). The oligonucleotides containing this sequence or random sequence were

subcloned into the lentiviral vector. For lentivirus production, 293FT packaging cells (Invitrogen) were transfected at 90% confluence by Lipofectamine 2000 (Invitrogen) with 12 μ g of lentiviral vectors, and the lentivirus-containing supernatants were harvested 48 h after transfection.

Cell culture

Brown preadipocytes were isolated from male C57BL/6J mice at the age of 3 weeks by collagenase digestion as described previously (Fasshauer *et al.* 2001, Klein *et al.* 2002, Tseng *et al.* 2004). Preadipocytes were grown to confluence in complete growth medium supplemented with 20 nM insulin (differentiation medium) (day 0). Adipocyte differentiation was induced by treating confluent cells for 48 h in differentiation medium further supplemented with 0.5 mM isobutylmethylxanthine and 1 mM cortisone. After this induction period (day 2), the medium was washed and cells were incubated in differentiation medium with additional 1 mM cortisone. The culture medium was changed every 2 days. Full differentiation was achieved after 6 days. BVT.2733, a selective inhibitor of 11 β -HSD1, was dissolved in 12% β -hydroxypropylcyclodextrin (Fluka) and added to the differentiation medium for 6 days during differentiation. For lentiviral infection of primary brown fat precursors, 70% confluent cell cultures were incubated with sh-11 β -HSD1 or scrambled shRNA or vector lentivirus (moi=100) overnight in complete growth medium. The medium was then replaced and cells were maintained in complete growth medium for an additional 24 h before inducing adipogenic differentiation.

Oil red O staining

After the induction of differentiation, cells were washed with PBS and fixed with 10% formalin in PBS for 1 h, washed three times with water, and finally air-dried. Cells were stained with oil red O (Sigma-Aldrich) (six parts of saturated oil red O dye (0.6%) in isopropanol and four parts of water) for 30 min. Excess stain was removed by washing with 70% ethanol. Stained cells were then washed with water and stored in PBS for visualization under the inverted microscope (Olympus).

Animals

Male C57BL/6J mice at the age of 3 weeks were purchased from Slac Laboratories (Shanghai, China). In

all experiments, the mice were housed three or four per cage in a room kept at $23 \pm 1^\circ\text{C}$ with a 12 h light:12 h darkness cycle and were allowed free access to water and food. From 3 weeks of age, mice were fed a normal fat diet containing 10% calories from fat (NC mice) (Collaborative Bio-Engineering Corporation, Nanjing, China) or a HFD containing 50% calories from fat (Collaborative Bio-Engineering Corporation) for 24 weeks. During the last 4 weeks, the HFD-fed mice were orally treated with 100 mg/kg BVT.2733 (an 11 β -HSD1 selective inhibitor was synthesized according to the patent information) at 0900 and 1700 h or vehicle. Body weight and food intake were determined weekly. At the time of killing, tissues were weighed, frozen in liquid nitrogen, and stored at -80°C . All animal studies were approved and experimental design approved and followed as per the Animal Care and Use Committee of Nanjing Medical University.

RNA preparation and quantitative real-time PCR

Total RNA was extracted from tissues using TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. Two micrograms of total RNA were reverse transcribed with 200 U Moloney murine leukemia virus reverse transcriptase (M-MLV RT; Promega), and in the presence of 0.5 mmol/l deoxynucleotide triphosphate, 25 U RNase inhibitor, and 0.5 μg N15 random primers, in a total volume of 25 μl . PCR primers were designed by Primer5 software. The sequences of the primers used are shown in Table 1. Each quantitative real-time PCR was carried out in triplicate in a 25 μl volume of SYBR Green Real-time PCR Master Mix (Toyobo, Osaka, Japan). The PCR program was designed as follows: 60 s at 95°C , followed by 40 cycles of 15 s at 95°C , 15 s at 60°C , 45 s at 72°C , and 5 s at 80°C on the plate reader (Rotor Gene-3000; Corbett Research, Sydney, Australia). All data were analyzed using the expression of the gene encoding β -actin as an internal reference. Expression for each gene is arbitrarily set at 1 to facilitate comparison between several groups.

Western blotting

For western blot analysis, cell or tissue extracts were lysed in RIPA buffer (0.5% NP-40, 0.1% SDS, 150 mM NaCl, and 50 mM Tris-Cl (pH 7.5)). Proteins were separated by 10% SDS-PAGE, transferred to PVDF membrane (Millipore, Massachusetts, United States), and probed with anti-uncoupling protein-1 (UCP1; Abcam, Hong Kong, China), anti-11 β -HSD1 (Abcam), anti- β -actin, and anti-tubulin (Abcam) antibodies.

Histology and immunostaining

Tissues were fixed in 10% formalin and were paraffin embedded. Multiple sections (5 μm) were prepared and stained with hematoxylin and eosin for general morphological observation. For immunostaining, the sections of brown fat tissues were incubated with anti-UCP1 antibodies (Abcam) for 30 min at room temperature. Cell cultures were fixed in 4% paraformaldehyde, followed by permeabilization in 0.3% Triton-X 100. Fixed cells were incubated with anti-UCP1 antibodies for 1 h at room

Table 1 Sequences of primers used

11 β -HSD1	Forward: 5'-AAG GAG CCG CAC TTA TCA GA-3' Reverse: 5'-TTC AAG GCA GCG AGA CAC TA-3'
Ucp1	Forward: 5'-AGG GTT TGT GGC TTC TTT TC-3' Reverse: 5'-TGG TTG GTT TTA TTC GTG GT-3'
Cidea	Forward: 5'-TCC TCG GCT GTC TCA ATG-3' Reverse: 5'-GGC TGC TCT TCT GTA TCG-3'
Cox7 α 1	Forward: 5'-AGG ACG CAA AAT GAG GGC-3' Reverse: 5'-TCT TGT GGG GGA AGG AGG-3'
Cox8b	Forward: 5'-GGA GTG CGA CCC CGA GAA T-3' Reverse: 5'-CGG CGG AAG TGG GAG TTT T-3'
mCPT1	Forward: 5'-ACC ACT GGC CGC ATG T-3' Reverse: 5'-CTC CAT GGC GTA GTA GTT GCT-3'
LCAD	Forward: 5'-GGA CTC CGG TTC TGC TTC CA-3' Reverse: 5'-TGC AAT CGG GTA CTC CCA CA-3'
Ucp3	Forward: 5'-CTG CCT GGA ACA GAA CAA AG-3' Reverse: 5'-GAC GAA ACA CGG AGG ACT AA-3'
Aco	Forward: 5'-ATG AAT CCC GAT CTG CGC AAG GAG C-3' Reverse: 5'-AAA GGC ATG TAA CCC GTA GCA CTC C-3'
PPAR α	Forward: 5'-CAA GGC CTC AGG GTA CCA CT-3' Reverse: 5'-TTG CAG CTC CGA TCA CAC TT-3'
PDK4	Forward: 5'-GGT TAC GGC TTG CCA ATT TCT CGT C-3' Reverse: 5'-TTG GGA TAC ACC AGT CAT CAG CCT C-3'
Perilipin	Forward: 5'-TGC TGG ATG GAG ACC TC-3' Reverse: 5'-ACC GGC TCC ATG CTC CA-3'
Adipophilin	Forward: 5'-AAG CAT CGG CTA CGA CGA CAC-3' Reverse: 5'-GGA CAG TCT GGC ATG TAG TCT GGA-3'
TIP47	Forward: 5'-GCT ATG GAG GAA CCT GTT GTG-3' Reverse: 5'-CGT GGA ACT GAT AAG AGG CAG-3'
Fsp27	Forward: 5'-GTG TCC ACT TGT GCC GTC T-3' Reverse: 5'-TGC TCG CTT GGT TGT CTT G-3'

temperature. Secondary detection was performed with goat anti-rabbit antibody (Abcam). Samples were visualized using a Nikon Eclipse 80i upright microscope (Nikon).

Electronic microscopy

Cellular samples were fixed for 1 h in a mixture of 2.5% glutaraldehyde, 1.25% paraformaldehyde, and 0.03% picric acid in 0.1 M sodium cacodylate buffer (pH 7.4); washed in 0.1 M cacodylate buffer; postfixed with 1% osmium tetroxide/1.5% potassium ferrocyanide for 1 h; washed in water; and stained in 1% aqueous uranyl acetate for 30 min followed by dehydration in grades of alcohol (5 min at 70%, 5 min at 90%, and 2, 3, and 5 min at 100%). The samples were then infiltrated and embedded in TAAB Epon (Marivac Canada, Inc., St Laurent, QC, Canada). Ultrathin sections (about 60 nm) were cut on a Reichert Ultracut-S microtome, picked up onto copper grids stained with uranyl acetate and lead citrate, and examined in a JEOL 2000FX (JEOL) microscope.

Measurements of O₂ consumption

Brown adipocytes were cultured in 24-well plates (Seahorse Bioscience, Massachusetts, United States). Four wells in each plate, in which no cells were plated, served as negative control. On day 6 post-differentiation, cells were switched to 500 μ l assay media containing 25 mM glucose, 2 mM GlutaMax-1, and 1 mM sodium pyruvate (Seahorse Bioscience) per well for measurement of oxygen consumption on an XF24 respirometer (Seahorse Bioscience). Oxygen consumption rates (OCR) were measured at basal glucose levels as well as with drugs disrupting the respiratory chain: oligomycin (ATP synthase inhibitor; 1 μ M) (Sigma-Aldrich) and FCCP (uncoupler; 4 μ g/ml) (Sigma-Aldrich).

Statistical analysis

Results are presented as mean $\pm$ S.E.M. Statistically significant differences were calculated using Student's *t*-test. A value of $P < 0.05$ was considered significant.

Results

Knockdown of 11 β -HSD1 promotes brown adipocyte function

Previously, we have found that expression of BAT-specific genes was significantly decreased in dexamethasone-treated mice (Supplementary Figure 1, see section on

supplementary data given at the end of this article). As 11 β -HSD1 functions as an activator of glucocorticoid synthesis, we hypothesized that modulating 11 β -HSD1 expression and/or activity could impact BAT function.

To this end, we probed the expression of 11 β -HSD1 during BAT differentiation. An inverse relationship between mRNA expression of 11 β -HSD1 (*HSD11B1*) and *UCP1*, a specific mitochondrial protein involved in the regulation of thermogenesis and energy expenditure in BAT, was observed during brown adipocyte differentiation (Fig. 1A and B), indicating that 11 β -HSD1 may serve as a genuine regulator of BAT function. To verify this hypothesis, short hairpin RNA (shRNA) targeting 11 β -HSD1 was introduced into primary brown fat pre-adipocyte by lentiviral infection to silence the expression of endogenous 11 β -HSD1 (Fig. 2A). As shown in Fig. 2B, mRNA levels of a host of BAT signature genes including *UCP1*, *Cidea*, *Cox7a1*, and *Cox8b* were significantly upregulated in 11 β -HSD1-depleted brown adipocyte

Figure 1

There is an inverse relationship between mRNA expression of 11 β -HSD1 and *UCP1* during brown adipocyte differentiation. (A and B) mRNA expression of *UCP1* and 11 β -HSD1 in primary brown adipocyte during differentiation was measured by quantitative RT-PCR. Results are representative of three independent experiments. Statistically significant differences between preadipocyte (d0) and differentiated cells (d2, d4, and d6) (* $P < 0.05$, ** $P < 0.01$) are indicated.

Figure 2

Knockdown of 11 β -HSD1 promotes brown adipocyte function. (A) Primary brown preadipocytes were transfected with scrambled (sh-control [sh-con]) or specific 11 β -HSD1 siRNA (sh-11 β -HSD1) overnight, and then after an additional 24 h in complete growth medium, cells were differentiated by exposure to differentiation medium for 6 days (medium was changed every 2 days). 11 β -HSD1 mRNA and protein levels of brown adipocyte were measured by quantitative RT-PCR and western blot. (B, E and F) The expression of genes related to brown fat function, FA oxidation, and lipid droplet proteins in brown adipocytes was determined by quantitative

RT-PCR. Results are representative of three independent experiments. Statistically significant differences between control and transfected cells are indicated. (C and D) Lipid droplet staining with oil red O was performed for gross photography (first row) and for microphotography (second row, original magnification, $\times 200$.) under microscope. Electronic microscopy analysis was performed on day 6 postdifferentiated cells. Note the difference in droplet size (red arrow). (G) Oxygen consumption rate was quantified by a Seahorse Biosciences XF 24 analyzer as described under Materials and methods section (* $P < 0.05$, ** $P < 0.01$).

compared with control cells. We also observed more UCP1 staining in 11 β -HSD1 knockdown adipocyte compared with control cells by immunohistochemistry analysis (Supplementary Figure 2, see section on supplementary data given at the end of this article).

To directly address whether the increased expression of brown fat functional genes in 11 β -HSD1 knockdown cells was due to improved differentiation of brown adipocytes, we measured the cellular lipid accumulation by oil red O staining. We found that both 11 β -HSD1 knockdown and control brown preadipocyte differentiated into mature adipocyte containing well-developed lipid droplets, showing indistinguishable variation in

gross morphology. Interestingly, 11 β -HSD1 knockdown cells exhibited significantly smaller lipid droplets than control cells as evident in inverted microscopy (Fig. 2C). Close examination using electronic microscopy analysis further demonstrated that the size of lipid droplets in mature brown adipocyte was significantly decreased in 11 β -HSD1 knockdown cells (Fig. 2D). It has been suggested that smaller lipid droplet size is associated with enhanced lipid oxidation and energy expenditure (Ellis *et al.* 2010). Indeed, consistent with reduced lipid droplet size, there was a significant increase in the expression levels of genes involved in FA β -oxidation, including CPT1a, LCAD (ACADL), PPAR α (PPARA), and UCP3 (Fig. 2E).

Within adipocyte, triglyceride is predominately stored within lipid droplets that are surrounded by a phospholipid monolayer containing various lipid droplet proteins, including perilipin, adipophilin, and TIP47 (PLIN3). Recently, Sawada *et al.* (2010) found that overexpression of perilipin in white fat was associated with increased FA oxidation and reduced lipid droplet size. Therefore, we examined the mRNA expression of lipid droplet proteins. Levels of perilipin, adipophilin, and TIP47 were increased in brown adipocyte in the absence of 11 β -HSD1, while the expression of FSP27 (CIDEC), a protein that promotes energy storage, was significantly decreased (Fig. 2F), indicating that 11 β -HSD1 may modulate the morphology of brown adipocyte by altering the expression of key proteins involved in lipid droplet formation.

Enhanced BAT function is typically associated with higher OCR. Therefore, we evaluated the cellular respiration by a Seahorse Bioscience XF24 respirometry analyzer. The data, as summarized in Fig. 2G, indicate that basal OCR was increased in brown adipocytes with 11 β -HSD1 depletion, consistent with enhanced BAT-specific gene expression. Furthermore, after the addition of an ATP synthase inhibitor (oligomycin/OL) or a chemical uncoupler (*p*-trifluoromethoxyphenyl hydrazone/ FCCP), the OCR of uncoupled respiration in HSD1-deficient adipocytes was still significantly higher than that in HSD1 normal adipocytes. The data indicate that 11 β -HSD1 plays a key role of a potential regulator of UCP1 in brown adipocyte oxygen consumption.

Overexpression of 11 β -HSD1 dampens brown adipocyte function

To directly evaluate the role 11 β -HSD1 plays in regulating brown adipocyte function and morphology, we overexpressed 11 β -HSD1 in primary brown adipocyte by means of lentiviral infection. As shown in Fig. 3A, mRNA and protein levels of 11 β -HSD1 were significantly increased in cells infected with the 11 β -HSD1 plasmid compared with cells infected with an empty vector. In the presence of ectopic 11 β -HSD1, mRNA levels of BAT-specific genes were downregulated consistent with the pattern observed in brown adipocyte depleted of 11 β -HSD1 (Fig. 3B). These observations were further confirmed by immunohistochemistry analysis that showed less UCP1-positive staining in 11 β -HSD1 overexpression adipocytes compared with controls (Supplementary Figure 3, see section on supplementary data given at the end of this article). Moreover, morphological examination also showed that 11 β -HSD1 promoted lipid storage as reflected by larger lipid droplet

size (Fig. 3C and D). The expression of FA β -oxidation genes and lipid droplet protein genes were also attenuated by 11 β -HSD1 overexpression (Fig. 3E and F). Of note, alterations in gene expression profile as well as in morphology of brown adipocyte induced by 11 β -HSD1 overexpression were abrogated by BVT.2733, a selective inhibitor of 11 β -HSD1. Meanwhile, changes in gene expression in the presence of exogenous 11 β -HSD1 were reflected by dampened oxygen consumption, which was alleviated by BVT.2733 (Fig. 3G). Collectively, these data indicated that augmented 11 β -HSD1 expression is associated with enlarged lipid droplet, accelerated fat storage, and attenuated energy expenditure.

Pharmacological inhibition of 11 β -HSD1 activity by BVT.2733 enhances brown adipocyte function

To further confirm the role of 11 β -HSD1 in brown adipocyte, primary murine brown preadipocyte was treated with the 11 β -HSD1 inhibitor BVT.2733. A dose- and time-dependent increase in UCP1 expression was observed in BVT.2733-treated cells (Fig. 4A, B and C; Supplementary Figure 4, see section on supplementary data given at the end of this article). In agreement with the results obtained in 11 β -HSD1 knockdown cells, a group of BAT-specific genes was preferentially activated by BVT.2733 (Fig. 4D). In addition, we also observed a significant increase in the number of multilocular lipid droplet with BVT.2733 treatment accompanied by upregulation of genes involved in β -oxidation (Fig. 4E, F and G). Finally, BVT.2733 treatment differentially regulated the expression of genes involved in lipid droplet formation. Those that promote a brown fat-like phenotype (adipophilin and TIP47) were selectively activated whereas those more closely associated with a white fat-like phenotype were selectively inhibited by BVT.2733 (Fig. 4H). In addition, treatment with BVT.2733 in BAT met a marked increase in oxygen consumption compared with mock-treated cells (Fig. 4I). Taken together, this line of data indicates that BVT.2733 treatment phenocopied 11 β -HSD1 silencing in brown adipocyte by promoting energy expenditure and reducing lipid accumulation.

BVT.2733 promotes BAT function in HFD-fed mice

To further validate the role for 11 β -HSD1 in brown adipocyte function and energy metabolism, we treated HFD-fed mice with BVT.2733 for 4 weeks. Compared with mice treated with vehicle, we observed a marked alleviation of obesity, rapid weight loss, and improved glucose

Figure 3

Overexpression of 11 β -HSD1 dampens brown adipocyte function. (A) Primary brown preadipocytes were transfected with 11 β -HSD1 expressing lentivirus (11 β -HSD1) or vector (con) overnight, and then after an additional 24 h in complete growth medium, cells were subsequently treated with BVT.2733 (200 μ mol/l) (HSD1 + BVT) for 6 days during differentiation (medium was changed every 2 days). 11 β -HSD1 mRNA and protein levels of brown adipocyte were measured by quantitative RT-PCR and western blot. (B, E and F) The expression of genes related to brown fat function, FA oxidation, and lipid droplets proteins in brown adipocytes was

determined by quantitative RT-PCR. Results are representative of three independent experiments. Statistically significant differences between control and transfected cells (* P <0.05, ** P <0.01) are indicated. (C and D) Lipid droplet staining with oil red O was performed for gross photography (first row) and for microphotography (second row, original magnification, $\times$ 200.) under microscope. Electronic microscopy analysis was performed on day 6 postdifferentiated cells. (G) OCR was performed by a Seahorse Biosciences XF 24 analyzer (* P <0.05, ** P <0.01 compared with control; # P <0.05, ## P <0.01 compared with 11 β -HSD1 overexpression group).

tolerance in mice treated with BVT.2733 (data not shown). In accordance, expression of BAT-specific genes including UCP1 was significantly upregulated (Fig. 5A and B; [Supplementary Figure 5](#), see section on supplementary data given at the end of this article). This was accompanied by a clear shrinkage in the size of brown fat cells (Fig. 5C). Fat burning was accelerated as evidenced by across-the-board activation of β -oxidation genes (Fig. 5D). In the meantime, expression of genes involved in lipid droplet formation was altered, resembling an activated brown fat-like phenotype. Therefore, our findings indicate that inhibition of 11 β -HSD1 by BVT.2733 could drive the activation of BAT and subsequently improve energy balance.

Discussion

11 β -HSD1 has been implicated as a key player in modulating WAT function by amplifying glucocorticoid activity (Morton 2010). 11 β -HSD1 null mice exhibited decreased white adipose depot, resistance to hyperglycemia induced by fat feeding, and increased hepatic insulin sensitivity (Alberts *et al.* 2002, 2003, Kershaw *et al.* 2005, Stewart & Tomlinson 2009). By contrast, increased 11 β -HSD1 activity in WAT resulted in the development of visceral adiposity, hyperglycemia, and insulin resistance in morbidly obese patients (Kannisto *et al.* 2004, Baudrand *et al.* 2010, 2011). Moreover, our previous studies suggested that 11 β -HSD1 could promote

Figure 4

Pharmacological inhibition of 11 β -HSD1 activity by BVT.2733 induces brown adipocyte function and metabolism in primary brown fat cells. (A and B) Concentration–response and time–response curves for the induction of mRNA expression of *UCP1* by BVT.2733 were measured by quantitative RT-PCR. Primary brown preadipocytes were cultured for 6 days in the vehicle (con) or in the presence of 200 μ M BVT.2733 (BVT.2733). (C) *UCP1* protein levels of brown adipocytes were measured by western blot. (D, G and H) The expression of genes related to brown fat function, FA oxidation, and lipid droplets proteins in brown adipocytes was determined by

quantitative RT-PCR. Results are representative of three independent experiments. Statistically significant differences between control and BVT.2733-treated cells (* P <0.05, ** P <0.01) are indicated. (E) Lipid droplet staining with oil red O was performed for gross photography (first row) and for microphotography (second row, original magnification, $\times 200$.) under microscope. (F) Electronic microscopy analysis was performed on day 6 postdifferentiated cells. (I) OCR was measured by a Seahorse Biosciences XF 24 analyzer (* P <0.05, ** P <0.01).

differentiation of white adipocyte *in vitro* (Liu *et al.* 2007). Thus, 11 β -HSD1 expression and its activity are clearly associated with a white fat-like phenotype *in vivo*.

Recently, Berthiaume *et al.* (2007) have reported that pharmacological inhibition of 11 β -HSD1 at a dose without affecting food intake could increase the mRNA level of *UCP1*, a BAT-specific marker gene. Li *et al.* (2012) have also found that administration of antisense 11 β -HSD1 in C57BL/6J mice could activate thermogenesis and enhance energy expenditure in BAT. However, the detailed maneuvering by 11 β -HSD1 to modulate BAT differentiation and/or function was not directly addressed in previous investigations. We here demonstrate that there was an inverse relationship between 11 β -HSD1 expression and *UCP1* expression during

brown adipocyte differentiation. Furthermore, we demonstrate that inhibition of 11 β -HSD1 either by BVT.2733 or by siRNA in brown adipocyte resulted in an increase in the expression of brown fat signature genes and enhanced oxygen consumption and fat burning. By contrast, overexpression of 11 β -HSD1 in antagonized brown adipocyte differentiation rendered a white fat-like phenotype. In addition, inhibition of 11 β -HSD1 activity by BVT.2733 phenocopied these *in vitro* observations in a mouse model of diet-induced obesity with the exception of COX8b, likely reflecting the differential dependence of individual genes on HSD1. Together, these results indicate that 11 β -HSD1 may drive up WAT differentiation and simultaneously suppress BAT differentiation.

Figure 5

Pharmacological inhibition of 11 β -HSD1 activity by BVT.2733 induces brown adipose tissue function and metabolism in high-fat diet (HFD)-treated mice. (A, D and E) HFD-fed mice were orally treated with BVT.2733 at 100 mg/kg (0900 and 1700 h) or vehicle for 2 weeks. The expression of genes related to brown fat function, FA oxidation, and lipid droplets proteins in interscapular brown fat pads was determined by quantitative RT-PCR in HFD-fed mice (con) and BVT.2733-treated mice (BVT.2733). Data are mean $\pm$ S.E.M. ($n=5-6$). Statistically significant

It is widely recognized that thermogenesis is primarily maintained through FA oxidation. During cold exposure, FA β -oxidation is simultaneously upregulated in BAT (Tseng *et al.* 2004). Hondares *et al.* (2011) recently reported that with the activation of PPAR α , the pivotal regulator for FA degradation in many organs, including in white adipocyte, led to the appearance of brown adipocyte as reflected by an increase in *UCP1* gene expression. Therefore, β -oxidation is a crucial function of BAT not only for energy dissipation but also for BAT phenotype maintenance. On the other hand, glucocorticoids are widely cited as being lipogenic within adipose tissue. It has been shown that enhanced glucocorticoid activity through overexpression of 11 β -HSD1 in WAT resulted in markedly decreased rate of fat oxidation and consequently central obesity (Candia *et al.* 2012). Our data link suppressed 11 β -HSD1 activity to accelerated FA degradation. Therefore, it is reasonable to postulate that containing 11 β -HSD1 levels *in vivo* may stimulate brown fat differentiation and function, antagonize white fat accumulation, and prevent obesity.

How does 11 β -HSD1 regulate brown fat cells toward β -oxidation? In adipocyte, triglyceride is predominately

stored within lipid droplets that are surrounded by a phospholipid monolayer containing various lipid droplet proteins, including perilipin, adipophilin, and TIP47. The established morphological studies found that brown adipocyte is characterized by the presence of multilocular lipid droplets in the cytoplasm, while white adipocyte has only one huge unilocular lipid droplet (Greenberg & Obin 2008). Recently, several reports have suggested a potential link between a specific expression profile of lipid droplet proteins and brown adipocyte morphology. For example, in animal models, overexpression of perilipin in white fat was associated with reduced lipid droplet size, which may induce BAT-like phenotype (Wang *et al.* 2012). Another study reported that knockout of FSP27, a protein localized in lipid droplets to promote lipid accumulation, induced a phenotype of obesity resistance, elevated oxygen consumption, extremely reduced WAT mass and white adipocyte size, and appearance of multilocular lipid droplets, all reminiscent of a brown fat-like phenotype (Liu *et al.* 2007). In addition, genes related to FA β -oxidation were also significantly increased in FSP27 knockout mice (Liu *et al.* 2007). Our results showed that inhibition of 11 β -HSD1 in brown adipocyte induced a

smaller lipid droplet size with corresponding changes in genes that contribute to lipid droplet formation.

Of note, 11 β -HSD1 is an enzyme that regulates the activity of glucocorticoids. Previously, we have found that the expression of BAT-specific genes was significantly suppressed in dexamethasone-treated mice (Supplementary Figure 1). A dose-dependent decrease in *UCP1* gene expression was observed in cells treated with either active glucocorticoid\dexamethasone or inactive glucocorticoid\cortisone (Supplementary Figure 6A and B, see section on supplementary data given at the end of this article). In addition, when cells were treated with RU486, the antagonist of glucocorticoid receptor, *UCP1* expression was increased (Supplementary Figure 6C and D). Of intrigue, the induction of *UCP1* gene expression by RU486 was much more prominent than by BVT.2733, indicating that 11 β -HSD1 may serve as a genuine regulator of BAT function by activating glucocorticoid and its receptor.

In summary, our data presented here point to a critical role for 11 β -HSD1 in regulating brown fat function, which may represent a promising therapeutic target for the treatment of obesity and metabolic syndrome.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/JME-12-0099>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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